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Transition of stable isotope ratios of leaf water under simulated dew formation

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ABSTRACT

Dew formation, a common meteorological phenomenon, is expected to intensify in the future. Dew can influence the $H_2{}^{18}O$ and HDO isotopic compositions of leaf water (δ_L), but the phenomenon has been neglected in many experimental and modelling studies. In this study, the dew effect on δ_L was investigated with a dark plant chamber in which dew formation was introduced. The $H_2{}^{18}O$ and HDO compositions of water vapour, dew water and leaf water of five species were measured for up to 48 h of dew exposure. Our results show that the exchanges of $H_2{}^{18}O$ and HDO in leaf water with the air continued in the darkness when the net $H_2{}^{16}O$ flux was zero. Our estimates of the leaf conductance using the isotopic mass balance method ranged from 0.035 to 0.087 mol m^{-2} s^{-1}, in broad agreement of the night-time stomatal conductance reported in the literature. In our experiments, the conductance of the C_4 species was 0.04 ± 0.01 mol m^{-2} s^{-1} and that of the C_3 plants was 0.10 ± 0.04 mol m^{-2} s^{-1}. At the end of 16 h dew exposure, 72 (±17) and 94 (±11)% of the leaf water came from dew according to the ^{18}O and D tracer, respectively.

Key-words: darkness; leaf water enrichment; photosynthesis; stomatal conductance; water isotopes.

INTRODUCTION

Dew formation is a common meteorological phenomenon in tropical and temperate climatic zones. Dew water can be an important hydrological input in some ecosystems. In a mid-latitude grassland ecosystem in the Netherlands, the amount of dew formation is about 4.5% of the mean annual precipitation and the frequency of dew nights is 70% (Jacobs *et al*. 2006). The frequency of dew occurrence is 84% over a growing season in an agricultural field in the midwest of USA (Welp *et al*. 2008). The long duration of dew presence, up to 14.5 h at the leaf surface of montane and subalpine plants in the central Rocky Mountains (Brewer & Smith 1997) and up to 22 h in the lower shaded canopy in tropical montane forests of Indonesia (Dietz *et al*. 2007), have been reported. In arid and semiarid regions, the role of dew formation in the regional water budget becomes more significant than in humid climatic regions. Dew relieves water stress by restoring 72% of leaf water at night in Mediterranean evergreen shrubs under constant water stress (Munne-Bosch, Nogues & Alegre 1999). Water input by night-time dew formation is equivalent to 11% of the annual precipitation in the desert valleys of the western USA (Malek, McCurdy & Giles 1999).

Dew formation also has important implications for studies of gas exchanges between the atmosphere and terrestrial ecosystems. The formation of dew is the result of radiation energy exchange between the cold plant surface and the atmosphere (Pitacco, Gallinaro & Giulivo 1992). Considering the dew flux in the energy balance budget provides a critical improvement to eddy covariance measurements which tend to overestimate evaporation component measurements made in the early morning hours (Sauer *et al*. 2007). Dew formation is highly effective in coating the leaf surface with liquid water (Burkhardt & Eiden 1994). The blockage of the stomata openings can alter the efficiency of diffusion of gases. The initiation of transpiration can be delayed in the early morning immediately after heavy dewfall (Pitacco *et al*. 1992). In the presence of dew water, the photosynthetic CO_2 uptake is decreased (Brewer & Smith 1997) because of 10 000 times slower CO_2 diffusion in water than in air (Weast 1977), except in cases of water-stressed plants where direct dew absorption can possibly improve photosynthesis (Munne-Bosch & Alegre 1999). From the experimental perspective, in the presence of dew water, it is nearly impossible to directly measure the leaf gas exchange of water vapour (Feild *et al*. 1998).

Dew formation can alter the $H_2{}^{18}O$ and HDO isotopic compositions of leaf water. In a study conducted in a soybean field, Welp *et al*. (2008) found that in the early morning the $^{18}O/^{16}O$ isotope ratio of leaf water in the upper canopy, which was covered with dew water, was in approximate equilibrium with water vapour, while the leaf water in the lower canopy, which remained dew free, was more positive than the isotopic equilibrium value. Their results suggest that the leaf stomata are not fully closed at night and may continue to exchange the minor isotopologues with the atmosphere even though there is no net water flux of the major isotope species. This process can be explained only by gaseous diffusion not by dew water penetration through the stomatal openings, because of the strong capillary force created by the stomatal geometry as well as the surface tension of liquid water (Schonher & Bukovac 1972).

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There is growing evidence that plant stomata remain partially open at night (Snyder, Richards & Donovan 2003; Ludwig, Jewitt & Donovan 2006; Barbour & Buckley 2007; Caird, Richards & Hsiao 2007b; Easlon & Richards 2009). A question of interest to us is whether plants can take up a significant amount of atmospheric vapour via the stomatal pathway in high-humidity conditions.

The occurrence of dew also impacts the leaf–air exchanges of the ^{18}O-CO_2 isotope. The oxygen isotope in CO_2 is exchanged with the ^{18}O-H_2O of leaf water by the enzyme reaction of carbonic anhydrase in leaves (Francey & Tans 1987). The dew alternation of ^{18}O-H_2O in leaf water, in turn, affects the ^{18}O-CO_2, which diffuses through open stomata back to the atmosphere without being assimilated (Farquhar & Lloyd 1993; Farquhar *et al*. 1993; Cernusak, Pate & Farquhar 2002). The retro-diffusion of ^{18}O-CO_2 is often assumed to be a half to two-thirds of CO_2 originally diffused into the leaf intercellular spaces (Francey & Tans 1987; Ciais *et al*. 1997); however, possible underestimation of the retro-diffusion has been discussed (Cernusak *et al*. 2004; Seibt, Wingate & Berry 2007). There may be time-delayed effects in the morning hours, influencing the ^{18}O-CO_2 that diffuses out of the stomata during photosynthesis. The dew alternation should be taken into account in predictions of global mean leaf water isotope ratios, which vary in a wide range of 0.4–6.5‰ (Farquhar *et al*. 1993; Ciais *et al*. 1997; West, Sobek & Ehleringer 2008).

In this study, we performed laboratory dew simulation experiments in order to examine the isotopic exchanges of leaf water with the vapour in the surrounding saturated air. We made continuous measurements of vapour isotope ratios in a dark plant chamber where saturation was maintained for up to 48 h by feeding with a vapour stream of known isotope ratios. In these experiments, the plant source water (or xylem water, δ_X) was isotopically much more enriched than the vapour feed, creating two unambiguous endmembers. This is in contrast to field conditions where the two endmembers are often isotopically indistinguishable (Welp *et al*. 2008; Wen *et al*. unpublished data). The artificially long dew exposure permitted the determination of the transient and steady-state behaviours of δ_L.

This study examines three hypotheses: (1) dew coating on the leaf surface enhances the leaf–air isotopic exchange of water; (2) the exchange of the minor isotopologues between the leaf and the surrounding air persists under the condition of no net flux of the major species; and (3) C_4 species are less likely to exchange water isotopologues in the leaf with the vapour even at very high humidity.

MATERIALS AND METHODS

Dew simulation experiment

An opaque polyethylene container (diameter: 0.5 m; height: 0.7 m; volume: 121 L) was used as the plant chamber. The chamber top was closed with a lid that did not form an airtight seal so air could continuously flow out of the chamber (Fig. 1). In this set-up, the air pressure inside the chamber was identical to the ambient atmospheric pressure. A layer of foam insulation outside the chamber stabilized the temperature inside, minimizing temperature fluctuations to less than 1 °C during each dew experiment. Two fans were installed inside the chamber to promote mixing.

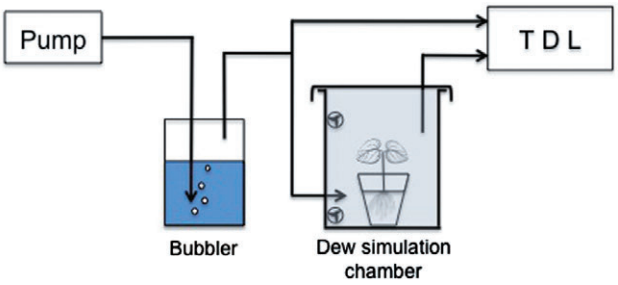

Figure 1. Schematic diagram of the dew simulation set-up consisting of a bubbler, dew simulation/plant chamber and a tunable diode laser (TDL) analyser for measuring water vapour isotopes. Arrows indicate the direction of airflow.

The chamber was fed with water vapour generated by a bubbler filled with water of known isotope ratios at an airflow rate of 3.5 L min^{-1}. As room air entered the bubbler reservoir, bubbles rose from an aquarium air stone at the end of air inlet near the bottom of the reservoir and produced a moist air stream. Because the moist air stream was saturated with respect to the reservoir temperature, its isotopic compositions could be predicted from the equilibrium fractionation factors, the reservoir temperature and its isotopic compositions (Majoube 1971). In the first dew experiment (wheat 1), the predicted values were –21.4 and –156.4‰ for $^{18}O/^{16}O$ and D/H, respectively. The vapour was gradually enriched as the experiments continued so that the predicted values in the last experiment (sorghum 3) were –14.7 and –108.6‰ for $^{18}O/^{16}O$ and D/H, respectively. The bubbler vapour stream was split into two: 3.25 L min^{-1} being sent to the plant chamber and a small portion (0.25 L min^{-1}) to a tunable diode laser (TDL) analyser (TGA 100; Campbell Scientific, Inc, Logan, UT, USA; Lee *et al*. 2005). The bubbler temperature was always set higher than the chamber temperature, ensuring dew formation inside the chamber. The vapour delivery tubing (Dekabon Type 1300, 1/4 in OD × 0.040 in wall; Dekoron, Aurora, OH, USA) was heated in order to prevent condensation inside.

The TDL analyser monitored, in real time, the isotopic compositions of the bubbler vapour (chamber inlet; δ_B) and the vapour inside the chamber (chamber outlet; δ_V). The air from the two intakes was mixed with dry air before entering the TDL sample cell to minimize instrument non-linearity (Lee *et al*. 2005). Because of this dilution, the mixing ratio measured with the TDL does not represent the true mixing ratio of the inlet and outlet air; instead, these mixing ratios were determined from the saturation vapour pressure at the chamber and bubbler temperatures.

The temperature and humidity of the chamber air were monitored by a thermocouple and a humidity probe (HMP45C-L; Vaisala Inc, Woburn, MA, USA) installed inside the chamber. Five plant species were chosen for

consideration of morphological and physiological traits. Of these plants, three were monocots with parallel venation (corn, *Zea mays*; sorghum, *Sorghum bicolor* (L.) Moench; and wheat, *Triticum durum*) and two were dicots with netted venation (soybean, *Glycine max*; cotton, *Gossypium hirsutum*). They also presented two types of photosynthetic system: C_3 (soybean, cotton and wheat) and C_4 (corn and sorghum). The plants were grown hydroponically in a greenhouse prior to the dew experiments. In the following, for convenience of presentation, we will refer to these species as C_3 and C_4 groups, but recognize that the species selected may not be representative of native plants.

Twenty-four hours prior to each experiment, the hydroponic solution of the experimental plants was replaced by a solution made of enriched water (0.4 to 6.0‰ for $^{18}O/^{16}O$ and –24.3 to 6.6‰ for D/H). This way, the plant xylem would have been fully flushed with the water of known isotope ratios by the time the experiment started. The experimental plants were exposed to full sunlight in the ambient environment for 3 h to produce enriched leaf water. After that, they were moved to the plant chamber which had been fed with the bubbler moisture for 48 h. The plant root system with the hydroponic solution was carefully sealed to avoid contamination of the chamber air from the evaporation of the solution. The isotopic ratio of the solution was measured at the beginning and the end of the experiment.

A total of 10 sets of complete dew simulation experiments were performed. Each experiment lasted 48 h and was repeated twice on every plant species. Such long dew exposure does not occur in field conditions, but it permitted more precise determination of the transient and steady-state behaviours of δ_L than shorter exposures.

Sampling of various water pools was conducted as the experiment progressed. Collection of leaf samples took place immediately before the plants were placed inside the chamber, and every 4–12 h afterwards until the end of the 48 h experimental period. Dew was first removed prior to leaf sampling using clean paper towels. Except for wheat, the main vein was removed before the leaf sample was archived for analysis, because the vein contained unfractionated xylem water. The dew water was collected at the end of the experiment. Because of leaf sample collection, the leaf area inside the chamber was reduced gradually over time over the dew simulation; this change was accounted for in the isotopic mass balance calculations (Eqn 8).

The leaf water was extracted using the cryogenic vacuum extraction method. The water isotopic analyses were carried out on a Thermo Finnigan DeltaPlus XP with Gas Bench and Thermo Finnigan MAT 253 with H-device in Yale University (New Haven, CT, USA).

Preliminary experiments

Prior to the dew simulation experiments described above, a pilot study was conducted with soybean and corn. During the pilot study, no measurement of δ_V was available. Two of the experiments were conducted in saturated conditions and another two under subsaturation (mean relative humidity inside the chamber = 89%). These experiments lasted 48 h. The goals of the pilot study were to test the experimental apparatus and logistics, and to compare the leaf water turnover in saturated and unsaturated conditions.

Isotopic mass balance

The method we used to estimate stomatal conductance (g_S) was based on isotopic mass balance principles. Firstly, we computed the net water flux of the chamber for the major and minor isotopologues. Then, we estimated the stomatal conductance of the plants inside the chamber. The complication introduced to the system of equations by dew formation inside the chamber was constrained by the measurement of dew water isotopic compositions and by the well-established relations of equilibrium fractionation.

The net water vapour flux of the plant chamber (F, mol m^{-2} s^{-1}) consists of dew flux (F_d) and leaf flux (F_L) as

$$F = F_d + F_L, \tag{1}$$

The molar ratio of the minor to major isotope species of the flux can be expressed as

$$R_F = \frac{F^i}{F} = \frac{F_d^i + F_L^i}{F_d + F_L}, \tag{2}$$

where superscript 'i' denotes the minor isotope species (^{18}O or D). At saturation, and because the leaf and air temperature were identical, the leaf flux F_L would vanish as there was no gradient in the $H_2{}^{16}O$ vapour pressure between the substomatal cavity and the chamber air. However, F^i_L was not zero because of the non-zero vapour pressure gradient of the minor isotopologue. Equation 2 can be simplified as

$$R_F = \frac{F_d^i + F_L^i}{F_d}, \tag{3}$$

and can be converted into the delta notation, as

$$\delta_F = \delta_d + (F_L^i / F_d) \times R_{std}^{-1} \times 10^3, \tag{4}$$

where δ_F and δ_d denote isotopic composition of chamber flux and dew condensation inside the chamber in per mil, respectively, and R_{std} is the standard VSMOW $^{18}O/^{16}O$ or D/H molar ratio.

Equation 4 was used to determine F^i_L from the measurement of all the other terms. The term δ_F on the left side of Eqn 4 was determined from the vapour isotopic composition δ_V (outlet) and δ_B (inlet), and saturation vapour pressure of the outlet and inlet airstreams at the chamber and the bubbler temperature, e_S (T_a) and e_S (T_B), as

$$\delta_F = \frac{e_S(T_B)\delta_V - e_S(T_a)\delta_B}{e_S(T_B) - e_S(T_a)}, \tag{5}$$

This equation is equivalent to the intercept of the linear relation between the vapour delta and the reciprocal of the

saturation vapour pressure. The term δ_d on the right side of Eqn 4 was given by

$$\delta_d = \delta_V + \varepsilon_{eq}(T_a), \quad (6)$$

$$\varepsilon_{eq} = (1 - \alpha_{eq}^{-1}) \times 10^3 \ (‰), \quad (7)$$

where α_{eq} (>1) is the temperature-dependent equilibrium fractionation factor (Majoube 1971) and ε_{eq} denotes the equilibrium fractionation factor in per mil. The equilibrium prediction of δ_d was used instead of the δ_d measured at the end of the experiment from the collected dew water, because δ_d was variable during the 48 h experiment. The dew flux, F_d, the rate of dew formation inside the chamber, was computed from difference in the saturation vapour density (ρ_S) at the chamber (T_a) and the bubbler temperature (T_B) as

$$F_d = Q\frac{\rho_S(T_a) - \rho_S(T_B)}{S}, \quad (8)$$

where S is leaf area (m^2) and Q is flow rate ($m^3\ s^{-1}$).

We note that F_L^i (mol $m^{-2}\ s^{-1}$), the leaf flux of the minor isotopes, is related to the stomatal conductance as

$$g_S = \frac{F_L^i}{e_S^i(T_a) - e_a^i} \times P_a, \quad (9)$$

where e_a^i is the vapour pressure of the minor isotope inside the chamber and P_a is atmospheric pressure (101.3 kPa). Here, e_a^i was obtained from the TDL measurement of δ_V and the chamber air temperature,

$$e_a^i = e_S(T_a)R_{std}\left(\frac{\delta_V}{10^3} + 1\right), \quad (10)$$

and e_S^i, the saturation vapour pressure of the minor isotope species at leaf temperature (T_a), is given by

$$e_S^i = \frac{e_S}{\alpha_{eq}}R_L, \quad (11)$$

where R_L is the molar ratio of the minor to the major isotope in the leaf water.

The estimate of g_S using Eqn 9 includes both stomatal and cuticular conductance; the latter is usually less than 0.02 mol $m^{-2}\ s^{-1}$ (Caird, Richards & Donovan 2007a). We also note that the g_S value is for the minor isotopologue, which is slightly (3%) lower than the value for the major isotopologue.

Response of leaf water isotope ratio to step change

The stomatal conductance estimate was used to quantify the time evolution of the leaf water isotope ratio (δ_L) under dew influence. The transient response of δ_L to the step change in the external forcing, which occurred when the plants were moved from the ambient condition to the plant chamber, is controlled by the turnover time of leaf water (τ). The isotopic composition of leaf water at a given time step is the mixture of unfractionated xylem water (δ_X) and the existing leaf water at the previous time step. Dongmann *et al*. (1974) suggested that the response follows the time course of change as

$$\delta_L = (\delta_{L,0} - \delta_{L,SS})\exp\left(\frac{-\Delta t}{\tau}\right) + \delta_{L,SS}, \quad (12)$$

where Δt is the time elapsed since the step change, and subscripts '0' and 'SS' denote the time of step change ($t = 0$) and steady state, respectively. In using Eqn 12 to predict δ_L, we assumed that the leaf water isotope ratios at the new steady state ($\delta_{L,SS}$) was equal to the isotopic composition of dew water (δ_d) given by Eqn 6. The τ value was determined from

$$\tau = \frac{W}{g_S\omega_i}, \quad (13)$$

where W represents leaf water content (mol m^{-2}) and ω_i is the mole fraction of water vapour in the intercellular space (Farquhar & Cernusak 2005).

RESULTS

Table 1 summarizes the results of the pilot study conducted on soybean and corn in the subsaturation and saturation conditions. The isotopic composition of the initial leaf water ($\delta_{L,0}$) was highly enriched over the xylem water immediately

Table 1. Summary of variables in the pilot study

	$\delta_{L,0}$ (‰)	$\delta_{L,48}$ (‰)	δ_X (‰)	δ_d (‰)	$\delta_{L,SS}$ (‰)	f
$^{18}O/^{16}O$						
Saturation						
Soybean	21.0	–7.2	2.6	–9.8	–7.3	0.75
Corn	24.9	–6.8	9.2	–10.4	–7.0	0.81
Subsaturation						
Soybean	10.2	–1.5	1.7	n/a	–1.5	n/a
Corn	19.6	0.2	3.0	n/a	0.1	n/a
D/H						
Saturation						
Soybean	13.9	–64.8	–19.8	–69.6	–65.0	0.90
Corn	39.6	–66.4	15.1	–72.3	–67.6	0.93
Subsaturation						
Soybean	0.4	–32.1	–16.7	n/a	–32.1	n/a
Corn	19.9	–24.7	–13.2	n/a	–25.2	n/a

Here, $\delta_{L,0}$ and $\delta_{L,48}$ are the measured leaf water isotope ratios at $t = 0$ and $t = 48$ h, respectively. The steady-state leaf water isotopic composition $\delta_{L,SS}$ was obtained by fitting Eqn 12 to the observed δ_L using a non-linear least square method. The endmember fraction (f) at steady state was computed with Eqn 14. If $f = 1$, 100% of the leaf water originates from dew water. δ_X is the xylem water isotopic composition and δ_d is the isotopic composition of the dew water estimated using the equilibrium theory for the liquid bubbler reservoir (δ_B).

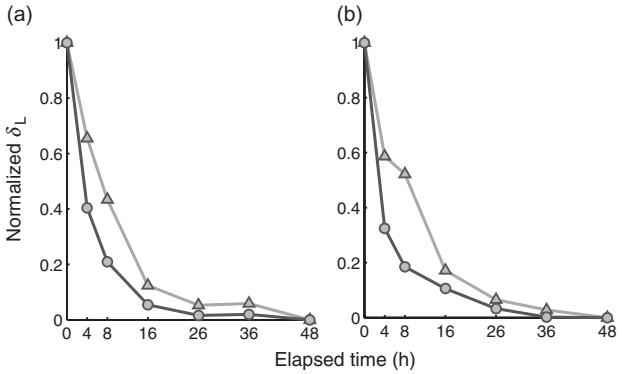

Figure 2. Normalized δ_L as a function of elapsed time during the pilot study for corn (triangles) and soybean (circles): (a) left panel, saturation; (b) right panel, subsaturation. The normalized δ_L values were the averages of the two isotopologues.

after the full sunlight treatment ($t = 0$), decreased steadily in the plant chamber until a new steady state was reached. For the saturation experiments, the leaf water isotopic composition at steady state ($\delta_{L,SS}$) was closer to the isotopic composition of dew water (δ_d) than to the xylem delta (δ_X). Here, the dew value was estimated from the equilibrium theory assuming that the vapour in the chamber was in equilibrium with the liquid reservoir of the bubbler at the bubbler temperature and that the dew water was in equilibrium with the water vapour at the chamber temperature. The relative role of the dew water can be quantified as a fractional contribution

$$f = (\delta_L - \delta_X)/(\delta_d - \delta_X), \quad (14)$$

This endmember fraction f is defined such that $f = 1$ indicates that all the leaf water originates from dew and $f = 0$ from the xylem water. The f value at steady state was 0.75 (soybean) and 0.81 (corn) according to the ^{18}O tracer, and 0.89 (soybean) and 0.94 (corn) according to the D tracer.

For the subsaturation experiments, the steady-state value $\delta_{L,SS}$ was 6.5‰ more enriched in ^{18}O and 37.7‰ in D than that obtained in the saturation experiments.

Figure 2 shows the transition of δ_L to the newer steady state during the pilot experiments. For convenience of comparison, δ_L was normalized as

$$\hat{\delta}_L = (\delta_L - \delta_{L,48})/(\delta_{L,0} - \delta_{L,48}), \quad (15)$$

and the normalized δ_L values of the two isotopologues were averaged in Fig. 2. Soybean showed faster transition to the steady state than corn in both saturation and subsaturation conditions. For example, under saturation, the soybean δ_L reached 80% of the full step change at 8 h, and at the same time, the corn δ_L approached about 60% of the full step change.

Figure 3 compares the predicted and the measured dew water isotopic composition for the 10 dew simulation experiments. This comparison serves as a check of the system consistency. The δ_d prediction was made using Eqn 6 with the chamber temperature and the vapour isotopic composition inside the chamber measured over the last 2 h of each experiment. Our predictions of the dew water isotope ratios are in excellent agreement with the measured values, with an average error of 0.29 and 3.0‰ for ^{18}O and D, respectively. The good agreement indicates that the TDL measurement was unbiased relative to the mass spectrometry results.

Figure 4 shows one example of the predicted δ_d and delta values of the measured water pools in one dew simulation experiment with corn. Table 2 lists the measurements of the water pools from all the 10 experiments reported as the average of two replicates for each species. The δ_d prediction (dashed line) was made using Eqn 6 with the δ_V measurement in reference to the chamber temperature. The predicted δ_d changed during the experiment in response to the changes in δ_V and approached the δ_d of the actual dew water collected at the end of the experiment (squares). This trend is seen in both $^{18}O/^{16}O$ and D/H. As in the pilot study, δ_L was highly enriched at $t = 0$ because of the full sunlight treatment, and slowly approached the new steady state. The observed δ_L did not return to isotopic composition of the plant source water at the new steady state. The plant source water changed very little over time (Table 2), indicating a negligible loss of the reservoir water by evaporation. Furthermore, the bubbler maintained a supply of vapour stream with relatively constant isotope ratios.

The chamber vapour delta was variable, but in a predicable way, over time. The δ_V spiked shortly after the experiment began and decreased continuously to a new steady state. Neither the bubbler nor the chamber temperature changes could have caused the initial increase of δ_V. The bubbler temperature fluctuated within ± 0.2 °C, which would produce a negligible change of 0.03 and 0.3‰ in the ^{18}O and D compositions, respectively, of the vapour entering the chamber. The chamber temperature immediately after the plants had entered showed an increase of

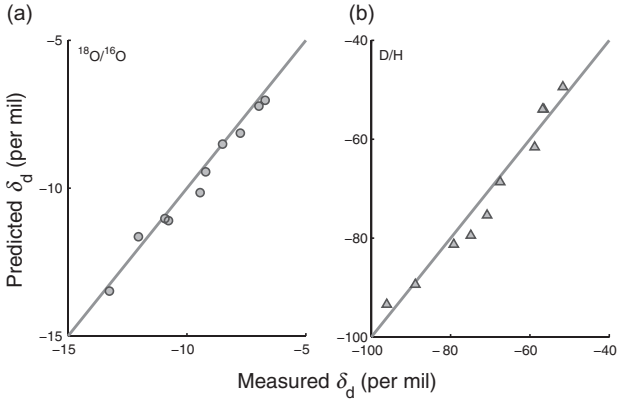

Figure 3. Comparison of the measured dew water isotopic composition and the equilibrium prediction according to Eqn 6. The solid lines represent a 1:1 relationship.

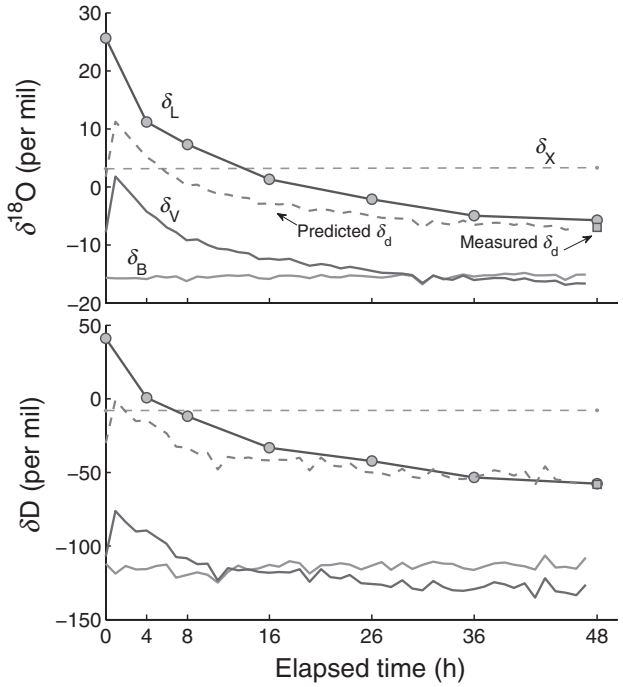

Figure 4. Time variations of the isotopic compositions of various water pools during a dew simulation experiment on a corn plant. The isotopic measurements of leaf water (δ_L, circles), dew water (δ_d, squares) and xylem water (δ_X, dots connected by dashed lines) were made with mass spectrometry. The measurements of the vapour in the chamber (δ_V) and the vapour generated by the bubbler (δ_B) were made with the tunable diode laser (TDL) analyser. The predicted dew delta value (δ_d, dashed) was obtained with the equilibrium relationship (Eqn 6).

0.78 ± 0.28 °C, and then stabilized from 3.3 h onwards. This initial increase was mainly caused by the heat released by the warm plants and the hydroponic solution brought in from the sunlight treatment. These temperature fluctuations produced a change of 0.06 and 0.75‰ in the isotopic equilibrium values for ^{18}O and D, respectively, not enough to explain the observed changes in δ_V. Instead, the change implies continued ^{18}O and D fluxes out of the leaves even under full saturation and the leaves being coated by dew water. This is because positive vapour pressure gradients of the minor isotopologues were maintained, by the highly enriched leaf water, between the substomatal cavity and the chamber air ($e_S^i - e_a^i > 0$ in Eqn 9). It is interesting to note that the trend of δ_V was almost identical to that of δ_L. In all the dew experiments, the flux of the minor isotopologues elevated the δ_V above the isotopic composition of the vapour entering the chamber (δ_B) for about 3 h after the onset of the experiment. Only towards the end of the experiments did δ_V become lower than δ_B. Our results indicate that the leaves maintained partially open stomata in the presence of dew coating on their surface.

Figure 5 compares $\delta_{L,SS}$ to the dew water and xylem water isotope ratios, each data point representing one dew simulation experiment. Here, $\delta_{L,SS}$ was determined by the regression fit of Eqn 12 to the observed δ_L. In the $\delta_d - \delta_{L,SS}$ plane (top *x*-axis and left *y*-axis, circles), $\delta_{L,SS}$ shows a strong linear relationship with δ_d that is parallel to the 1:1 line, indicating that δ_d played a significant role in determining $\delta_{L,SS}$ regardless of species or vein morphology. On the other hand, $\delta_{L,SS}$ shows much weaker correlation with δ_X (bottom *x*-axis and right *y*-axis, squares), indicating that the contribution of δ_X to $\delta_{L,SS}$ was minor.

Table 3 summarizes the results on the endmember fraction *f* (Eqn 14) at 16 h of dew exposure using measured δ_L at $t = 16$ and δ_d computed as the equilibrium value with δ_V measured at $t = 16$. The τ values in Table 3 are the means of the first 16 h measurements according to Eqn 13. The isotopic turnover time varied with the isotopologue and the photosynthetic mode. In general, the C_3 plants and the D isotope showed more rapid changes than the C_4 plants and the ^{18}O isotope. For the C_3 plants, the mean values of the turnover time for the first 16 h were 1.5 and 1.0 h for ^{18}O and D, respectively. For the C_4 plants, these values were 3.0 and 2.4 h for ^{18}O and D, respectively. The mean τ values were significantly different between the C_3 and the C_4 plants

Table 2. Isotopic compositions of leaf water (δ_L), plant source water (δ_X) and dew water collected at the end of the experiments (δ_d)

Elapsed time (h)	δ_L (‰) 0	4	8	16	26	38	48	δ_X (‰) 0	48	δ_d (‰) 48
$^{18}O/^{16}O$										
Corn	20.7	6.8	5.0	−1.6	−4.6	−6.7	−7.6	1.8	2.0	−8.9
Sorghum	17.5	6.5	1.0	−1.8	−4.0	−4.7	−5.6	5.4	5.4	−7.3
Wheat	14.1	2.1	−2.3	−5.2	−7.7	−8.6	−9.0	3.2	3.3	−10.9
Soybean	20.7	3.2	−1.9	−5.7	−7.6	−8.4	−8.6	2.4	2.5	−10.1
Cotton	18.4	2.4	−2.5	−6.6	−8.3	−9.2	−9.9	3.4	3.4	−10.7
D/H										
Corn	29.9	−15.3	−22.4	−45.9	−56.6	−67.2	−71.6	−16.1	−16.1	−71.5
Sorghum	25.3	−5.2	−27.4	−39.8	−48.0	−50.8	−53.5	4.6	4.1	−58.2
Wheat	13.7	−30.4	−49.0	−61.6	−72.5	−77.0	−80.1	−11.5	−10.7	−81.1
Soybean	26.0	−31.6	−49.5	−63.6	−72.8	−74.7	−78.5	−20.8	−20.9	−76.7
Cotton	18.5	−34.1	−51.4	−67.7	−78.0	−82.6	−87.2	−10.5	−10.0	−83.5

The values are reported as the average of two replicates for each species.

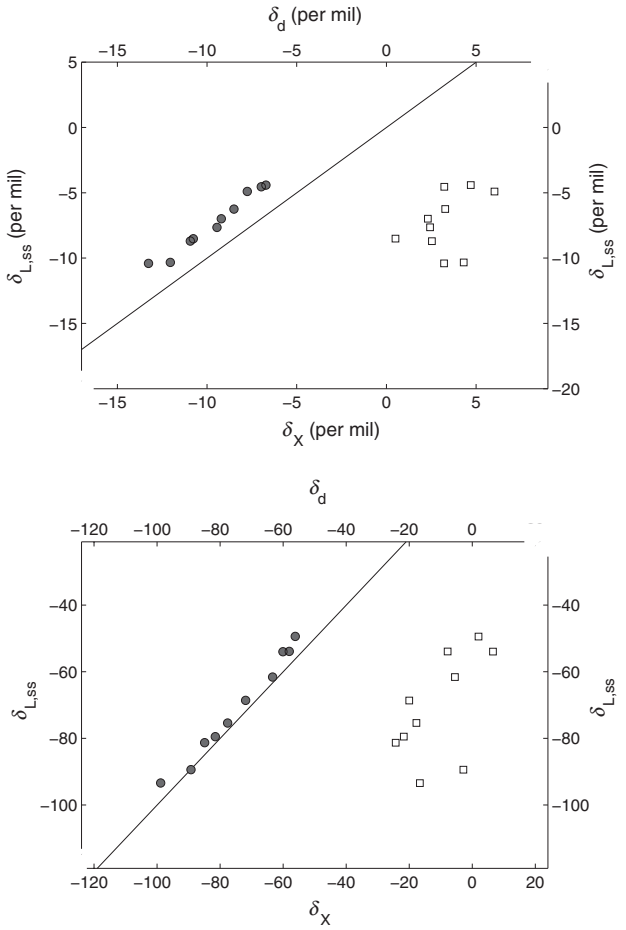

Figure 5. Comparisons of isotopic compositions of leaf water at steady state ($\delta_{L,SS}$, left *y*-axis) against that of the dew water collected at the end of each experiment (δ_d, circles; top *x*-axis) and $\delta_{L,SS}$ (right *y*-axis) against that of the xylem water (δ_X, bottom *x*-axis; squares). The top panel is for $^{18}O/^{16}O$ and bottom for D/H. The solid lines represent a 1:1 relationship.

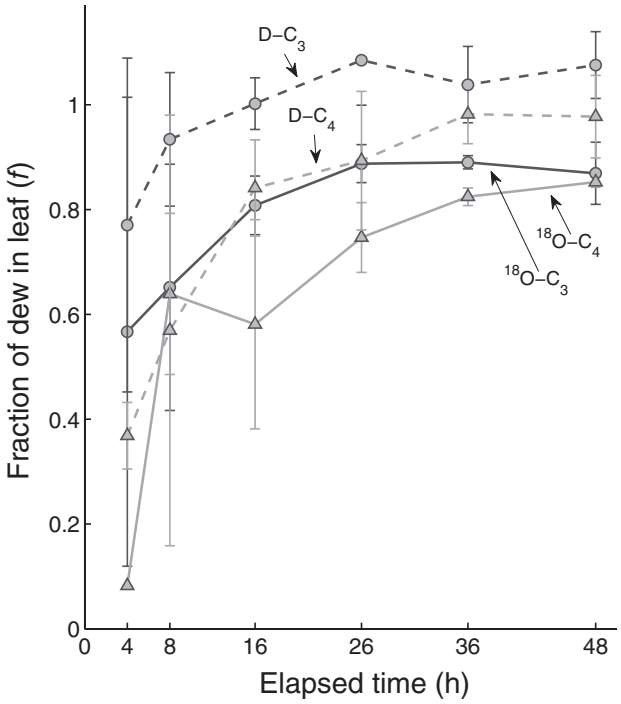

Figure 6. Fractional contribution of dew water to leaf water as a function of dew exposure time according to Eqn 14. The *f* values represent the averages of C_3 (circles; wheat, soybean and cotton) and C_4 plants (triangles; corn and sorghum) at a given time step according to the ^{18}O (solid lines) and D (dashed lines) tracer, respectively. Error bars indicate ± one standard deviation.

($P < 0.001$). Because our experiments were conducted with a limited sample size, further investigation would be needed to answer the question as to whether this difference holds for other species.

Table 3 and Fig. 6 indicate that the stomatal pathway played a large role in the temporal dynamics of δ_L in high-humidity conditions at night. The average *f* value at 16 h indicates that the dew contribution to the leaf water was 72% (±17%) and 94% (±11%) according to the ^{18}O and D tracer, respectively. Some dependence on plant species and photosynthetic mode was suggested by the data in Table 3 and Fig. 6. For the C_3 plants, the *f* value ranged from 0.73 to 0.90, and from 0.91 to 1.29 according to the ^{18}O and D tracer, respectively. For the C_4 plants, the *f* values were smaller, ranging from 0.31 to 0.79, and from 0.75 to 0.94 according to ^{18}O and D, respectively. The estimated *f* values greater than 1 were caused by uncertainties of the δ_V measurements with the TDL analyser.

Table 3. The endmember fraction (*f*) at $t = 16$ and the leaf water turnover time (τ) using the ^{18}O and D tracers

	$^{18}O/^{16}O$		D/H	
	τ (h)	*f*	τ (h)	*f*
Corn	2.7	0.45	2.3	0.76
Sorghum	3.2	0.72	2.5	0.92
Wheat	1.2	0.76	0.9	0.96
Soybean	1.8	0.82	1.1	1.05
Cotton	1.4	0.85	1.0	1.00

Note that if $f = 1$, all the leaf water originates from the dew water.

Figure 7 presents the stomatal conductance (g_S) estimated with the mass balance method at hourly intervals. According to the ^{18}O tracer, the estimated g_S was slightly lower than 0.02 mol m^{-2} s^{-1}, considered as a threshold cuticular conductance (Caird *et al.* 2007a), for soybean (Fig. 7c) and sorghum (Fig. 7e) at $t = 0$. At other times, g_S was greater than this threshold, indicating that the stomata were partially open in darkness. Use of the D tracer gave highly variable g_S values; occasionally, g_S would be negative, which is not an acceptable result. In comparison, the result obtained with the ^{18}O tracer was much more robust. For all the species, the g_S value estimated with the ^{18}O tracer had a minimum at the beginning and showed a slight increase with duration of dew exposure. The mean g_S of all the experiments was 0.036 mol m^{-2} s^{-1} at $t = 0$ and

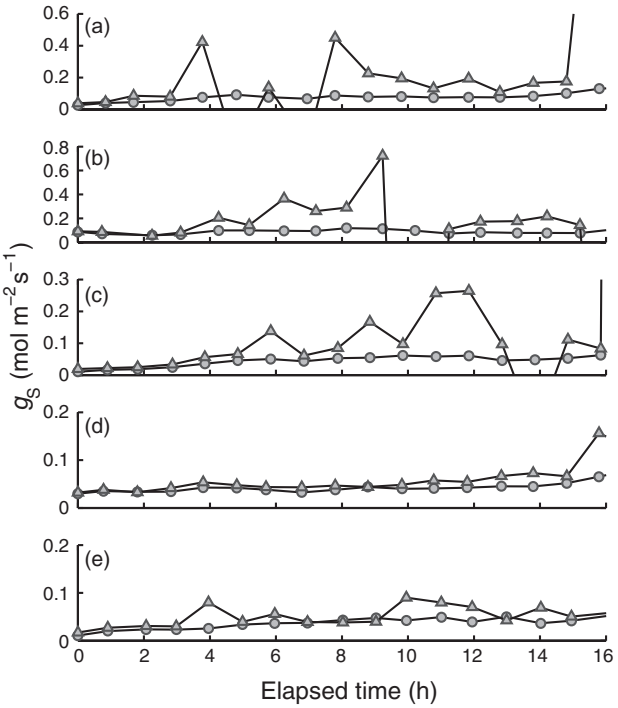

Figure 7. Stomatal conductance (g_S) determined with the $^{18}O/^{16}O$ (circles) and D/H (triangles) mass balance approach. Each data point represents an average of two replicates for each species: (a) cotton, (b) wheat, (c) soybean, (d) corn and (e) sorghum. For reference, the threshold cuticular conductance is about 0.02 mol m^{-2} s^{-1} (Caird *et al*. 2007a).

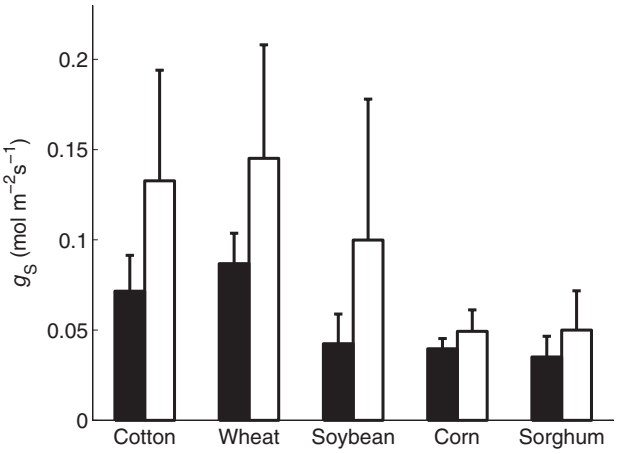

Figure 8. Comparison of the stomatal conductance for five experimental species (C_3: cotton, wheat and soybean; C_4: corn and sorghum) determined with the $^{18}O/^{16}O$ (filled) and D/H (open) tracer. The values represent 16 h averages and the error bars are standard deviations of the hourly values.

0.087 mol m^{-2} s^{-1} at 16 h. The increasing trend could be a result of increasing leaf water content and leaf water potential. In a separate experiment of corn plants growing in a well-watered field but exposed to ambient conditions, we found that the pre-dawn leaf water potential was 0.33 MPa higher in the presence of dew than plants in dew exclusion treatment. (Dew exclusion was achieved by suspending a piece of plastic over the treatment plot at night (Kim 2011).

Some variation of g_S among the plant species was suggested by the data in Fig. 8. The estimated g_S for the C_3 plants (cotton, wheat and soybean) were greater than that of the C_4 plants (corn and sorghum). According to the ^{18}O mass balance method, the mean g_S of the C_3 plants was 0.067 mol m^{-2} s^{-1} and that of the C_4 plants was 0.037 mol m^{-2} s^{-1}. Among the C_3 species, wheat showed larger values (mean 0.087 mol m^{-2} s^{-1}, ^{18}O tracer) than cotton (0.072 mol m^{-2} s^{-1}) and soybean (0.042 mol m^{-2} s^{-1}). The mean g_S of the C_4 plants was 0.040 mol m^{-2} s^{-1} (corn) and 0.035 mol m^{-2} s^{-1} (sorghum).

Figure 9 presents a comparison of the time variation of the measured δ_L with the predicted δ_L according to Eqn 12 for the same experiment described in Fig. 4. The predicted δ_L shows excellent agreement with the measured δ_L during the first 4 h of the experiment. After then, the predicted δ_L values were lower than the measurements for both isotopologues. In the case of D, the δ_L prediction was made by excluding periods when the estimate g_S was unreasonable (negative or greater than 0.5 mol m^{-2} s^{-1}). A similar disagreement was also obtained during other dew experiments. An implicit assumption of Eqn 12 is that the δ_L asymptotic approach to a new steady state is caused by a

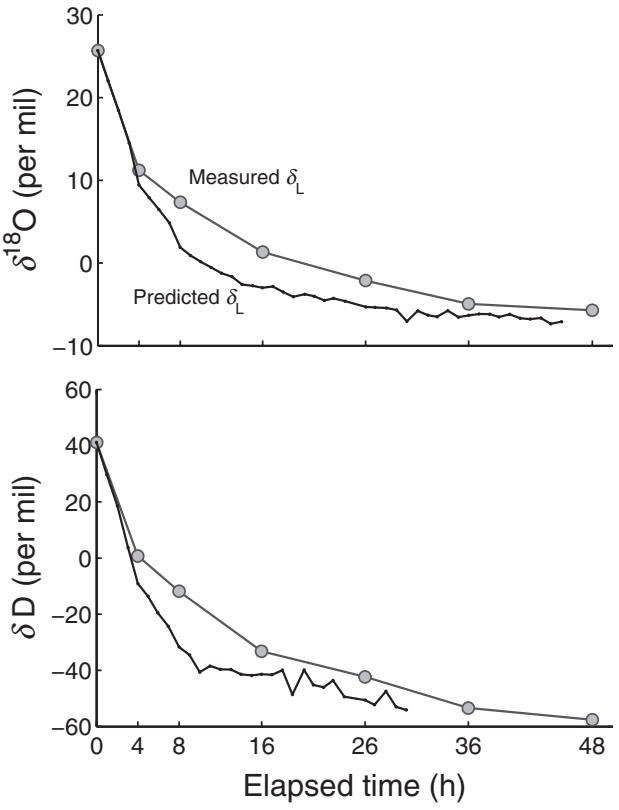

Figure 9. Comparison of the time variations in measured δ_L (grey circles) and predicted δ_L (black dotted lines, Eqn 12) using the hourly δ_V measurements for the corn plant described in Fig. 4.

step change in the external forcing. This assumption was not satisfied in our experiments. A sharp change to the driving variables of the plant gaseous exchange occurred when the plant was moved from sunlight to the dark enclosed chamber. However, the continued isotopic exchange through the partially open stomata modified the chamber vapour isotope ratios (δ_V) inside the chamber (Fig. 4).

DISCUSSION

Nocturnal isotope exchange at high humidity

Our measurements of δ_V reveal that a considerable degree of night-time isotope exchange occurs between the air and leaves at extremely high humidity. In Fig. 4, δ_V showed a steep increase immediately after the plant (corn) was placed in the chamber from full sunlight. The δ_V value inside the chamber was higher than δ_B for 30 h in the case of $^{18}O/^{16}O$, and 10 h in the case of D/H. For comparison, the turnover time of the chamber air was about 0.6 h. That $\delta_V - \delta_B$ was positive indicates a net flow of the $H_2{}^{18}O$ and HDO molecules from the leaf, whose water was enriched in ^{18}O and D, to the surrounding air whose vapour isotopic compositions were much lower. The positive gradient occurred in spite of dew formation which would make the vapour inside the chamber lighter than the vapour entering the chamber. It provides qualitative but direct evidence that the plants maintained partially open stomata in darkness and with the presence of dew water.

The complete absence of transpiration was observed on a night in the presence of heavy fog (Burgess & Dawson 2004) and at zero vapour pressure deficit (Daley & Phillips 2006; Dawson *et al*. 2007). In the presence of dew water on the leaf surface, we also expect zero vapour pressure deficit and absence of transpiration. Our results indicate that under these conditions, the net flux of the minor isotopologues may not be zero. The theoretical analysis of Farquhar & Cernusak (2005) also shows that incomplete stomatal closure permits molecular diffusion exchange of the minor isotopes of the leaf water with atmospheric vapour while the net flux of $H_2{}^{16}O$ can be zero.

Implications of dew occurrence for leaf water delta

Our data show that the exchange of $H_2{}^{18}O$ and HDO in the leaf water with the vapour was not hampered by the dew presence on the leaf surface. Dew water is highly effective in coating plant tissues (Burkhardt & Eiden 1994; Brewer & Smith 1997; Washington, Cruz & Fajardo 1998). Because it spreads evenly on both the abaxial and adaxial sides of the leaf, dew water may interfere with stomatal gaseous exchange of both amphistomatous and hypostomatous plants. It is a common practice in leaf gas exchange studies to ignore diffusion through the stomata of wet leaves on the assumption that surface water films act as an impermeable barrier to gas diffusion (Wesely 1989). The validity of the assumption remains unknown because conventional methods cannot measure the stomatal conductance of a wet leaf. Our data show that the assumption is not valid for ^{18}O-H_2O and HDO diffusion.

The incomplete closure of stomata was observed for all the plant species investigated in this study. The high *f* value suggests that the stomatal pathway played a large role in the temporal dynamics of δ_L in the darkness and high-humidity conditions. It is well established that evaporation through fully open stomata drives the isotopic enrichment of leaf water, resulting in maximum enrichment in the afternoon when transpiration is the highest diurnally. At night, the enriched leaf water is supposedly diluted by the unfractionated xylem water if leaves maintain complete closure of stomata. Because of long duration of our experiments, mixing with the xylem water would have caused δ_L to approach the xylem water isotope ratios. Instead, as a consequence of incomplete stomatal closure, the diffusion exchange with atmospheric vapour brought δ_L closer to δ_d or roughly the value in equilibrium with atmospheric vapour. Evidence of atmospheric vapour control on δ_L has been documented both in laboratory and in natural field conditions (Helliker & Griffiths 2007; Reyes-Garcia *et al*. 2008; Welp *et al*. 2008; Hartard *et al*. 2009; Helliker 2011; Wen *et al*. unpublished data).

According to the ^{18}O tracer, the fractional contribution of dew water to the leaf water did not reach unity at steady state (Table 3; Fig. 6), indicating some diffusive exchange of the leaf water with the xylem water. As plants usually undergo water stress during the daytime, sap flow at night exists in order to recharge water storage in leaves (Barbour *et al*. 2005; Daley & Phillips 2006; Caird *et al*. 2007b). In our experiments, the plants did not have water stress even after high transpiration demands during the full sunlight treatment because they were grown in the hydroponic system. The fractional contribution of the xylem water varied from 0.06 to 0.20 among the experiments.

The *f* value at 16 h was on average 15–22% lower for $^{18}O/^{16}O$ than that for D/H at steady state except corn (Table 3; Fig. 6). In other words, the fractional contribution of the xylem water to the leaf water ^{18}O pool was 15–22% higher than to the D pool. This was largely a result of difference in the efficiency of molecular diffusion in liquid water as diffusion should be a main driving factor to govern the movement of the minor isotopologues because of the elimination of the flux of the major isotope species in our experiment. These differences broadly agree with the differences between molecular diffusion of two isotopologues in liquid. In liquid water, HDO diffuses 14% more slowly than $H_2{}^{18}O$ at 25 °C (Wang, Robinson & Edelman 1953). Although mixing of the leaf and xylem water existed, the large *f* value shows that this mixing mechanism was not as efficient as the exchange with atmospheric vapour.

The δ_L temporal dynamics observed during the dew events raise the hypothesis that leaf water uptake occurs through direct infiltration of liquid dew water at the leaf surface. The possibility of the direct foliar uptake was suggested as plant adaptation to dry environments (Burgess & Dawson 2004). In the time course of the dew events,

stomatal conductance tended to increase gradually in all the five species (Fig. 7), which may be a result of direct uptake of dew through the stomata. However, the direct uptake of dew water through the stomatal openings would have to overcome the high surface tension of liquid water associated with the geometry of the stomata (Schonher & Bukovac 1972). An alternative hypothesis is to consider the role of dew as an intermediary. Dew preserves the influence of atmospheric vapour by maintaining an isotopic equilibrium state with the latter. Diffusion of the $H_2{}^{18}O$ and HDO molecules takes place in the gaseous phase through the stomatal opening that connects the dew water to the internal leaf water pool. Thus, although the transpiration flux vanishes in leaves covered by dew, the flux of the minor isotopes is potentially large. In fact, the isotopic flux has been shown to cause detectable changes in the isotopic content of atmospheric vapour (Welp *et al*. 2008).

Stomatal conductance of wet foliage

It is not possible to measure the stomatal conductance of wet foliage with conventional leaf chambers because of the difficulty in measuring water vapour exchange in high-humidity conditions. In this research, we have demonstrated that the stable isotopes of water provide a unique way to overcome this difficulty. This is possible because the exchange of the minor isotopes continues to occur with wet foliage. In our experiments, we deliberately used heavier water as the plant source water and lighter water to generate water vapour. The large isotopic difference between these two water pools helped to break the net isotopic flux into the two component fluxes: the downward exchange with dew water and the upward xylem water flow. Exposure to sunlight, together with the heavy xylem water, produced highly enriched leaf water relative to the vapour fed into the chamber at the beginning of the experiment. The large vapour pressure gradient of the minor isotopologues between the leaf and the air (Eqn 9) produced measureable isotopic flux signals, which then permitted the determination of the stomatal conductance.

In principle, the isotopic mass balance method can also be applied in field conditions, regardless of the leaf wetness status. It is now feasible to measure the isotopic fluxes with micrometeorological methods. The isotopic fluxes can be used with measurements of the leaf, vapour and dew water isotopic compositions, to determine the canopy conductance, in a similar manner to our laboratory set-up described in the Dew Simulation Experiment section. One challenge is that the measurements of evapotranspiration isotope flux tend to be noisier at night than in the daytime (Lee, Kim & Smith 2007; Welp *et al*. 2008; Griffis *et al*. 2010). Furthermore, in field conditions, the xylem and dew water isotopic compositions are often indistinguishable.

Our results add to a growing amount of evidence suggesting that incomplete stomatal closure is common in darkness for both C_3 and C_4 species (Barbour *et al*. 2005; Caird *et al*. 2007b). Across the species, our conductance values (0.035–0.15 mol m^{-2} s^{-1}) fall in the range of the night-time conductance values previously reported for herbaceous annual species (Snyder *et al*. 2003; Barbour *et al*. 2007; Caird *et al*. 2007a,b; Easlon & Richards 2009). The C_4 plants (corn and sorghum) had smaller conductance than the C_3 plants (cotton, wheat and soybean), implying that the C_4 plants may have developed a conservative stomatal behaviour by minimizing the night-time stomatal opening even at saturation humidity (Fig. 8). Among the C_3 species, wheat showed the largest values of g_S. It is not known whether the largest g_S of wheat among the three C_3 species was related to its parallel leaf venation. According to the ^{18}O tracer mass balance method, the C_4 species had mean stomatal conductance values of 0.040 mol m^{-2} s^{-1} (corn) and 0.035 mol m^{-2} s^{-1} (sorghum). The occurrence of non-zero night-time conductance of C_4 plants is not accepted widely, because the adaptation of the C_4 photosynthetic pathway to the environments with low water availability would mean the development of complete stomatal closure to avoid unnecessary water loss. That corn and sorghum maintained partially open stomata in darkness may have been a result of lack of water stress in our experiments. Corn and sorghum are two common domesticated C_4 species, which have been widely cultivated for energy industry and food supply. Future experiments with an expansion of species including C_4 grasses should provide improved generalization on the C_3/C_4 comparison in understanding the nocturnal stomatal behaviours. In field conditions, dew formulation can also relieve the physiological drought stress by increasing the leaf water potential (Kim 2011), which may induce stomata to open.

CONCLUSIONS

Our dew simulation experiments showed that leaf water continued to exchange the ^{18}O-H_2O and HDO molecules with atmospheric vapour in saturation humidity conditions when there was no net transpiration. The role of dew appeared to be one of an intermediary: it preserved the influence of atmospheric vapour by maintaining an isotopic equilibrium state with the latter. Diffusion of the $H_2{}^{18}O$ and HDO molecules took place in the gaseous phase through the partially open stomata that connected the dew water to the internal leaf water pool. In dew events, both dew water and xylem water contributed to the leaf water in darkness. The fractional contribution (f) of dew water to the leaf water at $t = 16$ was 0.81 ± 0.06 and 1.00 ± 0.05 for the C_3 plants, and 0.58 ± 0.20 and 0.84 ± 0.09 for the C_4 plants, according to the $^{18}O/^{16}O$ and D/H tracer, respectively. These high f values indicate that the exchange with atmospheric vapour was the dominant process controlling δ_L at saturation air humidity. These values likely represent an upper limit for natural ecosystem because natural dew events are mostly shorter than 16 h (Fig. 6). The lower f values for ^{18}O than for D indicated a more efficient diffusive exchange of ^{18}O between the leaf water and the xylem water. The average isotopic turnover time fell in the range of 0.9–1.8 h for the C_3 plants, and 2.3–3.2 h for the C_4 plants.

We used the mass balance approach to determine the stomatal conductance of the wet foliage. According to the ^{18}O isotopic mass balance method, the stomatal conductance varied from 0.035 to 0.087 mol m^{-2} s^{-1} among the five species; these values fell in the range of the values of the night-time stomatal conductance reported in the literature. Prolonging the exposure to dew caused g_S to increase slightly, suggesting that dew water may have increased the leaf water potential. (The D isotopic mass balance method was less reliable as the estimated g_S was very noisy and occasionally negative.) Of the species used in this study, the C_4 stomatal conductance tended to be lower than the C_3 conductance, and further investigation including C_4 grasses will be needed in order to draw a firm conclusion.

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